Volume 204, number 2 FEBS 3921 August 1986

The activity of γ-glutamyltranspeptidase in regenerating rat liver

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Received 12 May 1986

γ-Glutamyltranspeptidase is expressed at low levels in the liver of the male Fischer 344 rat where it exhibits 15-fold purification and 33% recovery in isolated plasma membranes. While the activity of the enzyme is unaltered in regenerating liver 24 h after partial hepatectomy, it increases steadily thereafter over a period of one week. Seven days after partial hepatectomy the enzyme is maximally activated: 5.6-fold in liver homogenates and 5.3-fold in isolated liver plasma membranes. The enzyme declines in activity over the next fourteen days and is expressed at normal levels three weeks after partial hepatectomy. These results demonstrate that the activity of γ-glutamyltranspeptidase increases in regenerating liver but that the increase is out of phase with the proliferative response.

Regeneration (Rat liver) γ-Glutamyltranspeptidase Hepatoproliferation

1. INTRODUCTION

The enzyme γ-glutamyltranspeptidase ([5-glutamyl]-peptide:amino-acid 5-glutamyltransferase, EC 2.3.2.2) catalyzes the transfer of the γ-glutamyl moiety of γ-glutamyl-containing compounds, notably glutathione, to acceptors including amino acids, dipeptides and glutathione itself (reviews [1,2]). It is widely distributed in nature [3,4] and it exhibits tissue-dependent differences in activity [1,5]. Relative to other tissues the activity in liver is extremely low, although we have shown striking species-dependent differences in activity of the liver enzyme [6]. Results from this laboratory [5,6] indicate that while γ-glutamyltranspeptidase is expressed at low levels in the liver of the adult rat, the enzyme is expressed at high levels in fetal/neonatal liver, premalignant liver and hepatomas. Others have also shown that γ-glutamyltranspeptidase activities are higher in developing liver, premalignant liver and liver tumors [7,8]. These results suggest that, in the rat, increased liver γ-glutamyltranspeptidase activity is associated with, and an indication of, hepatoproliferative states. To test this hypothesis further, the present study examines in the male Fischer 344 rat the activity of γ-glutamyltranspeptidase in regenerating liver which is a widely used model of liver proliferation.

2. MATERIALS AND METHODS

Male Fischer 344 rats (170–175 g body wt) were obtained from Canadian Breeding Laboratories, Montreal, Canada. They were housed 10–12 per cage in large stainless steel cages, fed regular rat chow, given water at libitum, maintained on a 12 h:12 h light/dark schedule at a temperature of 21°C and humidity of 60%. They were acclimatized for a period of two weeks before the start of the experimental protocol.

Analytical or reagent grade chemicals were obtained from either Sigma, St. Louis, MO or Fisher Scientific, Saskatoon, Canada.

2.1. *Preparation of animals*

58 male Fischer 344 rats (200–220 g) were used for these studies. Four rats were used for preliminary analysis of the activity of liver γ-

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00145793/86/$3.50

glutamyltranspeptidase. 36 rats were partially hepatectomized; 9 rats were sham operated and served as operated controls; 6 rats were not surgically treated and served as non-operated controls.

Partial hepatectomies involving removal of the three main liver lobes were performed under light ether anesthesia by the standard method of Higgins and Anderson [10]. Sham operations were also carried out under light ether anesthesia and involved opening the abdominal cavity and manipulating the liver gently. To avoid the influence of circadian rhythm, all surgery was performed and all liver samples were obtained between 8.30 a.m. and 10.00 a.m. All rats were killed by decapitation at the indicated time intervals.

Partially hepatectomized rats were sacrificed at exactly 24 h (n = 11), 48 h (n = 4), 96 h (n = 4), 120 h (n = 3), 7 days (n = 6), 14 days (n = 4), and 21 days (n = 4) after surgery. Sham-operated control rats were killed 24 h (n = 3), 7 days (n = 3) and 21 days (n = 3) after surgery. Non-operated control rats were killed immediately before the start (n = 3) and immediately after the end (n = 3) of the protocol which spanned 25 days.

2.2. *Preparation of liver homogenates*

After decapitation, animals were bled and livers removed quickly and placed in ice-cold homogenizing buffer (0.25 M sucrose, 10 mM Tris-Cl, 0.5 mM DTT, 0.5 mM $CaCl_2$, pH 7.4). Livers were trimmed of connective tissue and fat. 1 g representative portions were minced finely with scissors in 4 vols homogenizing buffer and homogenized in a Dounce homogenizer with an 'A' pestle using 40 strokes. After filtering through cheese cloth, homogenates were dispensed into storage tubes and stored for not more than 2 weeks at −80°C.

2.3. *Isolation of plasma membranes*

Plasma membranes were prepared by a procedure outlined in detail [5] from 4 of each of the following livers: non-operated control liver, 24 h regenerating liver, and 7 day regenerating liver. Briefly, 1 g portions of liver were homogenized exactly as described, filtered through cheese cloth and centrifuged at 10000 × g in an IEC B-20A preparative centrifuge at 4°C for 12 min. The resulting pellets were resuspended in homogenizing buffer (1 ml/g liver) with the Dounce homogenizer (8 strokes; A pestle). The suspensions were brought to 46.5% (w/w) with 69% sucrose (w/w, in 10 mM Tris-Cl, 0.5 mM DTT, pH 7.4) as determined by a refractometer. These were dispensed into ultracentrifuge tubes overlaid with an equal volume of 41% sucrose (w/w, in 10 mM Tris-Cl, 0.5 mM DTT, pH 7.4) and centrifuged in an IEC B-60 ultracentrifuge at 4°C at 110000 × g for 75 min in an IEC SB-283 swinging bucket rotor.

The plasma membrane enriched fractions, which were located at the top of the gradient, were removed by Pasteur pipette, suspended and diluted in 40 ml of 10 mM Tris-Cl, 0.5 mM DTT, pH 7.4, and centrifuged at 40000 × g in an IEC B-20 preparative centrifuge at 4°C for 40 min. The plasma membranes were suspended in 10 mM Tris-Cl, 0.5 mM DTT, pH 7.4, using a ground glass Duall homogenizer to a protein concentration of 2–2.5 mg/ml, dispensed into storage tubes and frozen at −80°C. γ-Glutamyltranspeptidase activity was determined the next day in carefully thawed samples.

2.4. *γ-Glutamyltranspeptidase assay*

γ-Glutamyltranspeptidase activity was determined in carefully thawed liver fractions with L-γ-glutamyl p-nitroanilide as substrate donor and glycyl-glycine as acceptor following the procedure of Naftalin et al. [11]. The assay mixture contained 4.6 mM L-γ-glutamyl p-nitroanilide, 100 mM glycyl-glycine, 100 mM Tris-Cl, pH 8.0, and 30–150 μg protein in a final volume of 500 μl. The reaction was started by the addition of substrate, incubation was carried out from 20–30 min at 37°C, and the reaction was terminated by the addition of 1.0 ml of ice-cold 10% glacial acetic acid. The enzymatically liberated p-nitroaniline was diazotized according to a modified Bratton-Marshall procedure. The absorbance of the azo dye thus generated (proportional to enzyme activity) was measured at 540 nm. Enzyme activity is expressed as nmol p-nitroaniline generated/min per mg protein.

Protein content of liver fractions was determined by the method of Lowry et al. [12] using crystalline bovine serum albumin as standard.

3. RESULTS

3.1. *The activity of γ-glutamyltranspeptidase in Fischer 344 rat liver*

As indicated in table 1, the activity of γ-glutamyltranspeptidase is very low in the liver of the Fischer 344 rat. Relative to homogenate, the enzyme exhibits a 15.1-fold purification and recovery of 33.4% in the plasma membrane-enriched fraction indicative of its plasma membrane locus.

3.2. *The activity of γ-glutamyltranspeptidase in regenerating liver*

The activity of γ-glutamyltranspeptidase in livers of control rats before and after the start of the 25 day protocol was not different. Also, liver γ-glutamyltranspeptidase activity was not different in sham-operated controls relative to non-operated controls at any point examined after surgery. Results from all control groups, therefore, have been combined to give a value for control rat liver homogenates of 0.213 ± 0.021 (n = 15) nmol·mg^{-1}·min^{-1}.

The activity of γ-glutamyltranspeptidase in homogenates of regenerating rat liver prepared at various times after partial hepatectomy is illustrated in fig.1. Compared to the activity of control liver, the activity in 24 h regenerating liver is not different. From 48 h to 7 days after hepatectomy, the activity of liver γ-glutamyltranspeptidase exhibits a steady increase. Relative to control, γ-glutamyltranspeptidase is increased 1.6-, 2.1-, 3.7- and 5.6-fold in livers regenerating, respectively, for 48 h, 72 h, 120 h and 7 days after hepatectomy. From a peak response at seven days post hepatectomy, liver γ-glutamyltranspeptidase activity exhibits a drop over the next 7 days. On day 14, the activity of liver homogenate γ-glutamyltranspeptidase is 2.2-fold that of control liver homogenate. By the 21st day after partial hepatectomy, the liver enzyme is expressed at control levels.

Table 1

γ-Glutamyltranspeptidase in the liver of the male Fischer 344 rat

	γ-Glutamyltranspeptidase		
	Specific activity (nmol·mg^{-1}·min^{-1})	Relative specific activity (SA PM/SA HOM)	Recovery (% from homogenate)
Homogenates (n = 4)	0.195 ± 0.024	15.2	33.4
Plasma membranes (n = 4)	2.964 ± 0.242		

The activity of γ-glutamyltranspeptidase was determined under standard conditions in homogenate and plasma membrane fractions isolated from 4 control Fischer 344 rat livers. Results represent mean values ± SE. Relative specific activity is derived from the specific activity of the plasma membrane fraction (SA PM) divided by the specific activity of the homogenate (SA HOM). Recovery represents the amount of γ-glutamyltranspeptidase recovered in the plasma membrane as a percentage of the total amount of the enzyme present in the homogenate

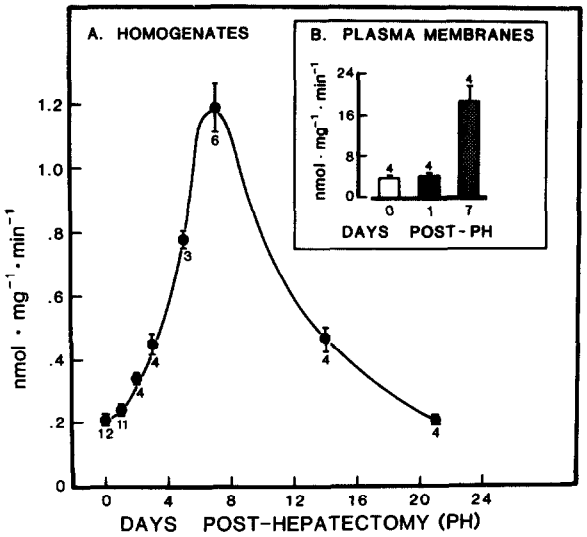

Fig.1. The activity of γ-glutamyltranspeptidase was determined under standard conditions in homogenates prepared from control Fischer 344 rat liver and in homogenates of Fischer 344 rat liver regenerating for various time periods after partial hepatectomy. (Inset) Activity of γ-glutamyltranspeptidase in control liver as well as 24 h and 7 day regenerating liver of the Fischer 344 rat. At each time point, results represent mean values ± SE for the indicated number of samples each prepared from the liver of a separate rat.

The results presented in the inset of fig.1 illustrate the activity of γ-glutamyltranspeptidase in plasma membranes isolated from control liver and regenerating liver 24 h and 7 days after partial hepatectomy. As is the case with homogenates, no differences are apparent 24 h after partial hepatectomy while large differences are observed 7 days after hepatectomy. γ-Glutamyltranspeptidase is increased 5.3-fold over control in 7 day regenerating liver plasma membranes.

The relative specific activities of γ-glutamyltranspeptidase are similar in control liver plasma membranes (15.9) and 7 day regenerating liver plasma membranes (14.8). The recovery of the enzyme in plasma membranes is also similar: 32.5% in the case of control liver, 33.4% in the case of 7 day regenerating liver. This indicates that similar purity and recovery profiles are exhibited by control and 7 day regenerating liver plasma membranes.

4. DISCUSSION

In accord with recent work from this laboratory [5,6] the present studies indicate that γ-glutamyltranspeptidase is expressed at very low levels in the liver of the Fischer 344 rat and that the enzyme in liver is largely plasma membrane bound.

The activity of the rat liver enzyme has been shown previously to be elevated in such hepatoproliferative states as developing, premalignant and malignant liver [7–9]. This study establishes for the first time that a significant elevation in enzyme activity occurs in regenerating liver which is a widely used model of liver proliferation. The time course of this induced increase in γ-glutamyltranspeptidase activity in regenerating liver is highly significant in terms of its relationship to hepatoproliferative events. In rats, it has been established (reviews [13–15]) that following hepatectomy, restorative hyperplasia occurs within the residual liver lobes, the proliferative peak of which occurs at approx. 24 h after surgery. During the next 48–72 h, proliferation wanes, as newly formed hepatocytes begin to differentiate and gradually recreate the acinar structure typical of mature liver. By 10 days, the process of regeneration is considered to be largely complete.

Against this sequence of changes, it is evident that γ-glutamyltranspeptidase activity increases out of phase with the proliferative response. It is clearly unaltered at 24 h during peak proliferation. It first rises 48 h after partial hepatectomy, is maximal 7 days after surgery and gradually returns to normal levels over the next 14 days. These results explain why others [16] have failed to observe significant changes in the activity of γ-glutamyltranspeptidase in regenerating liver since they have examined time intervals from 24 to 120 h after partial hepatectomy. These studies therefore establish that although increased γ-glutamyltranspeptidase activity is associated with the hepatoproliferative state of liver regeneration it is not coincident with the hepatoproliferative response. In this context, it is important to point out that the pattern of changes in the activity of γ-glutamyltranspeptidase in regenerating liver does, in fact, closely resemble the pattern of changes exhibited by this enzyme during its normal ontogenetic development. Results from this laboratory [5] as well as the results of others [7,8] have shown that although γ-glutamyltranspeptidase activity is very high in fetal liver, the high activity persists and actually peaks in the immediate postnatal period at a time when proliferation has ceased and hepatocyte differentiation predominates.

Whether or not the increase in hepatic γ-glutamyltranspeptidase which occurs in association with liver proliferation is an integral part of the proliferative response or is a consequence of it is under further study in this laboratory.

ACKNOWLEDGEMENT

This work was supported by a grant from the Saskatchewan Health Research Board.

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